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Iron and colorectal cancer risk in the α-tocopherol, β-carotene cancer prevention study

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***In vitro* and *in vivo* studies have associated iron with both the initiation and promotional stages of carcinogenesis. We investigated whether iron was associated with colorectal cancer in a nested case-control study within the α-tocopherol, β-carotene cancer prevention study cohort. Exposure was assessed at baseline, using a 276-item food frequency questionnaire and a fasting serum sample. The study included 130 colorectal cancer cases (73 colon cancers and 57 rectal cancers) and 260 controls. Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). Supplemental iron intake was only reported for 4 cases and 18 controls; therefore, we were unable to obtain meaningful results for this variable. Comparing the highest to the lowest quartiles, there was an inverse association between serum ferritin and colorectal cancer risk (*OR* = 0.4, 95% *CI* = 0.2–0.9) and a suggestion of an inverse association between dietary iron and colorectal cancer risk (*OR* = 0.4, 95% *CI* = 0.1–1.1). In addition, serum ferritin, serum iron and transferrin saturation were all inversely associated with colon cancer risk specifically (*OR* = 0.2, 95% *CI* = 0.1–0.7, *p* trend = 0.02; *OR* = 0.2, 95% *CI* = 0.1–0.9, *p* trend = 0.05; *OR* = 0.1, 95% *CI* = 0.02–0.5, *p* trend = 0.003, respectively), whereas serum unsaturated iron binding capacity was positively associated with colon cancer risk (*OR* = 4.7, 95% *CI* = 1.4–15.1, *p* trend = 0.009). In summary, we found a significant inverse association between several serum iron indices and colon cancer risk.**

Key words: red meat; iron; colorectal cancer

There is increasing epidemiological evidence implicating red meat as a risk factor for colorectal cancer, whereas white meat has not been associated with this cancer.[1] One of the main differences between red and white meat is the higher iron content in red meat.

There are many mechanisms through which iron might influence the carcinogenic process, particularly with respect to colorectal cancer. Iron is able to catalyze the formation of reactive oxygen species, which can induce oxidative DNA damage.[2] In addition, rodent studies have shown that iron is associated with increased crypt cell proliferation in the large intestine[3] and an increased rate of tumor growth in chemically-induced colorectal cancer.[4,5] Heme iron specifically, which is mainly found in meat, has been associated with increased cytotoxicity of fecal water from rodents,[6] increased endogenous formation of carcinogenic *N*-nitroso compounds[7] and promotion of chemically-induced colorectal cancer in rats.[8]

Of the 3 large cohort studies to address the association between iron and colorectal cancer, one examined dietary iron intake and found a significant positive association between iron consumption and cancer of the proximal colon.[9] All 3 cohorts examined iron stores and colorectal cancer risk; one cohort found increased risks for both colon and rectal tumors[9] and one found an increased risk for rectal cancer in women only,[10] but the third cohort observed no association between iron stores and colorectal cancer risk.[11] Furthermore, several case–control studies have been conducted to determine the effect of iron on colorectal cancer risk; a review of 33 of these studies, weighted according to their design and number of subjects, concluded that both higher dietary iron and iron stores were associated with an increased risk of colorectal cancer in the strongest studies.[12]

Although earlier epidemiological studies have investigated dietary or biochemical iron status in relation to colorectal cancer, no single study has simultaneously assessed dietary iron, supplemental iron and serum markers of iron status with respect to colorectal cancer risk. Thus, the aim of this study was to investigate these three iron exposures in relation to colorectal cancer.

Material and methods

Study population

We conducted a nested case-control study within the α-tocopherol, β-carotene cancer prevention (ATBC) study. The design of the ATBC study has been described in detail elsewhere.[13] Briefly, the ATBC study was a randomized, double-blinded, placebo-controlled, 2 × 2 factorial design, prevention trial to determine whether α-tocopherol and/or β-carotene supplements could reduce the incidence of cancer. The study recruited 29,133 Caucasian, male smokers, aged 50–69 years from south-western Finland between the year 1985 and 1988. Exclusion criteria included those who smoked fewer than 5 cigarettes per day, had prior cancer, a serious disease limiting long-term participation, or if they took vitamins E, A or β-carotene supplements in excess of predefined doses. During the two clinic visits prior to randomization, all participants completed a questionnaire on lifestyle factors and a 276-item food frequency questionnaire (FFQ).[14] In addition, a fasting serum sample was collected and then stored at −70°C. The trial was concluded in 1993, but postintervention follow-up has continued through the Finnish Cancer Registry, which provides almost 100% case-coverage.[15] The study was approved by the institutional review boards of the National Cancer Institute and the National Public Health Institute of Finland, and written, informed consent was obtained from all participants.

Case ascertainment

This analysis included histologically confirmed colorectal cancer cases, diagnosed after the first 5 years of follow-up (to reduce the likelihood of preclinical cancer influencing diet or serum indices) through 30th April 2002, and who did not have any of the fol-

Abbreviations: ATBC, α-tocopherol, β-carotene cancer prevention study; CI, confidence interval; CRP, C-reactive protein; FFQ, food frequency questionnaire; OR, odds ratio; TIBC, total iron binding capacity; UIBC, unsaturated iron binding capacity.

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lowing: family history of colorectal cancer (at least one or more first-degree relative), any other malignant disease, ulcerative colitis or Crohn's disease. Medical and pathology records were collected from the hospitals and laboratories and reviewed by two study oncologists to confirm the colorectal cancer diagnosis. In the ATBC cohort, there were 449 colorectal cancer cases diagnosed after the first 5 years of follow-up through April, 2002; of these, 28 had a cancer previously, 5 were anal cancers, 1 had a missing serum draw date and, therefore, could not be adequately matched to a control, 1 case serum was broken in transit and 284 did not have a sufficient amount of serum available due to selection for studies of other hypotheses, leaving 130 colorectal cancer cases for analysis. Matched controls were selected from participants in the ATBC study, who were alive at the time the case subject was diagnosed and free from cancer (except nonmelanoma skin cancer). Controls were matched (2:1) to the cases by age (± 5 years), study area, intervention group and month of baseline blood draw (to control for seasonal variation of nutrient intake and sample degradation). Subsite-specific colorectal cancer risks were also determined for the colon (ICD-9 codes 153.1–4, 153.6 and 154.0) and rectum (ICD-9 code 154.1), separately.

Dietary assessment

The FFQ aimed to assess the usual frequency of consumption of foods over the past 12 months; it was used with a portion size booklet of foods, each with 3–5 different portion sizes. The FFQ was linked to a food-composition database of the National Public Health Institute in Finland, to estimate intake of dietary and supplemental iron as well as the intake of potential enhancers (*e.g.* meat) and inhibitors (*e.g.* alcohol, fiber and calcium) of iron absorption. Dietary information was available for 117 cases and all 260 controls.

Serum iron assessment

Serum ferritin, an indicator of the amount of iron stored in the body and a strong correlate of heme iron intake, was measured using a immunoradiometric assay (Count-A-Count Ferritin IRMA: Diagnostic Products Los Angeles, CA). Serum iron and unsaturated iron binding capacity (UIBC) were measured using a standard ferrozine-based iron colormetric assay (Olympus AU 400e auto analyzer). Total-iron-binding capacity (TIBC) was calculated as the sum of serum iron plus UIBC and is a measure of the iron-binding capacity within the serum and reflects the availability of iron-binding sites on transferrin. Transferrin saturation was calculated as serum iron divided by TIBC. Transferrin saturation indicates the extent to which transferrin has vacant iron-binding sites (*i.e.* low transferrin saturation indicates a high proportion of vacant iron-binding sites). Since inflammation is associated with both body iron stores and colorectal neoplasia,[16] we measured serum C-reactive protein (CRP) as a potential confounding variable of the association between iron and colorectal cancer risk. CRP levels were measured using a latex particle enhanced immunoturbidimetric assay kit (K-ASSAY CRP Ultra (Equal Diagnostics, Exton, PA)).

Serum samples were analyzed blinded to case-control status, and 47 aliquots (12%) of quality control serum were inserted into the sample batches. Based on the latter samples, the coefficient of variation for all analytes was less than 6.4%.

Statistical analysis

The distributions of baseline characteristics in cases and controls were compared using Wilcoxon rank sum tests for continuous variables and χ^2 tests for categorical variables. Spearman correlation coefficients were used to determine correlations between the iron variables. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression. ORs are reported for quartiles (with cut-points based on control data), using the first quartile as the referent category. Tests for linear trend were calculated using the median value of each quartile. All reported *p* values are two-sided. Potential confounding variables were investigated and the following were included in the multivariate models: age (continuous), education (junior high school and above versus less), body mass index (continuous, kg/m^2), number of years smoked, total number of cigarettes per day, physical activity (active—moderate or heavy occupational or recreational activity versus inactive), total caloric intake (kcal/day), alcohol (g/day of ethanol), ever daily aspi-

TABLE I – BASELINE CHARACTERISTICS OF THE STUDY POPULATION[1]

Characteristic	Cases (n = 130)	Controls (n = 260)	p-value[2]
Age (years)	56 (53–61)	57 (53–59)	0.37
Body mass index (kg/m^2)	26.0 (24.0–28.8)	26.1 (23.7–28.3)	0.65
Total cigarettes/day	20 (13–25)	20 (15–25)	0.53
Years smoked	36 (30–41)	36 (31–40)	0.94
Physically active (%)	34 (26.2)	59 (22.7)	0.45[3]
Educated (% junior high school or above)	34 (26.2)	55 (21.2)	0.27[3]
Ever daily use of aspirin (%)	8 (6.2)	51 (19.6)	0.002[3]
Dietary intake (daily)			
Total energy (kcal)	2756 (2230–3377)	2767 (2242–3250)	0.81
Carbohydrate (g)	309 (234–362)	300 (245–363)	0.81
Protein (g)	101.3 (82.6–121)	101 (85–121)	0.79
Fat (g)	99 (81–131)	103 (77–125)	0.80
Fiber (g)	24 (18–33)	37 (22–56)	0.58
Alcohol (g)	16 (4–29)	13 (4–25)	0.45
Calcium (mg)	1324 (983–1633)	1331 (1042–1659)	0.62
Beef (g)	23 (13–41)	21 (12–38)	0.58
Fish (g)	34 (22–51)	37 (22–56)	0.44
Poultry (g)	8 (0–19)	9 (0–20)	0.25
Sausage (g)	67 (39–88)	61 (38–91)	0.89
Pork (g)	36 (25–52)	37 (26–51)	0.65
Organ meat (g)	3.9 (0.8–8.0)	3.4 (0.0–6.8)	0.15
Dietary iron (mg)	18 (14–23)	19 (14–22)	0.88
Supplemental iron use (%)	4 (3.1)	18 (6.9)	0.12[3]
Serum markers			
CRP (mg/l)	3.4 (1.7–6.5)	2.6 (1.4–4.8)	0.04
Ferritin (ng/ml)	136 (76–202)	141 (88–221)	0.29
Iron (μg/dl)	117 (91–145)	124 (97–153)	0.28
Transferrin saturation (%)	36 (28–44)	38 (30–47)	0.15
TIBC (μg/dl)	330 (303–354)	326 (300–351)	0.35
UIBC (μg/dl)	207 (176–232)	200 (167–230)	0.16

[1]All values are medians (interquartile range) unless otherwise indicated.–[2]*p*-values derived from the Wilcoxon rank sum test unless otherwise indicated.–[3]*p*-value derived from the χ^2 test.

rin use and serum CRP level (continuous, mg/l). In addition, meat, folate, fruit and vegetable intake and vitamin supplement use did not confound the relation between iron and colorectal cancer risk and were, therefore, not included in the final regression models.

We also stratified our analyses by cancer site (colon versus rectum) and by the median value (based on controls) of dietary factors purported to affect iron absorption including (*e.g.* alcohol, fiber, calcium, meat, vitamin C) as well as factors thought to affect iron indices (*e.g.* follow-up time, median in cases, serum CRP, median in controls).

All statistical analyses were carried out using statistical analytic systems (SAS, version 8.2) software (SAS institute, Cary, NC).

Results

The median follow-up in the combined 130 colorectal cancer cases (73 colon, 57 rectal) and 260 controls was 14.2 years (range, 1.6–17 years). There were two notable differences in baseline characteristics between cases and controls: 20% of the controls reported ever daily use of aspirin compared to just 6% of cases, and mean serum CRP in controls was 2.6 mg/l compared to 3.4 mg/l in cases (Table I).

All indices, including dietary and serum iron measures, were found to be in the normal range expected for older men. Dietary iron was not correlated with any of the serum iron indices (Table II), although supplemental iron intake was correlated with both serum iron and transferrin saturation ($r = -0.29$ and $r = -0.26$, respectively). Serum ferritin was higher in those who consumed above the median amount of red meat when compared with those who consumed less than the median (median values of 148 ng/ml compared to 134 ng/ml, respectively), although this difference was not significant ($p = 0.16$).

Only 4 cases and 18 controls, which equates to 3.1% of cases and 6.9% of controls, reported taking supplemental iron; therefore, no further results are reported for supplemental iron. Comparing the highest to the lowest quartiles, there was a suggestion of an inverse association between dietary iron and colorectal cancer risk (*OR* = 0.4, 95% *CI* = 0.1–1.1, *p* trend = 0.06) and a significant inverse association between serum ferritin and colorectal cancer risk (*OR* = 0.4, 95% *CI* = 0.2–0.9, *p* trend = 0.09) (Table III).

TABLE II – CORRELATION MATRIX FOR DIETARY AND SERUM IRON INDICES

Variable	Serum iron	Transferrin saturation	TIBC	UIBC	Dietary iron	Iron supplements	Serum CRP
Serum ferritin	0.17	0.24	−0.19	−0.29	−0.06	−0.08	0.09
p-value	0.0009	<0.0001	0.0002	<0.0001	0.29	0.48	0.08
Serum iron		0.94	0.29	−0.63	−0.04	−0.29	−0.20
p-value		<0.0001	<0.0001	<0.0001	0.43	0.01	<0.0001
Transferrin saturation			−0.03	−0.85	−0.04	−0.26	−0.19
p-value			0.62	<0.0001	0.46	0.02	0.0002
TIBC				0.50	−0.002	−0.06	−0.08
p-value				<0.0001	0.97	0.61	0.10
UIBC					0.03	0.16	0.10
p-value					0.59	0.18	0.04

TABLE III – AGE-ADJUSTED AND MULTIVARIATE ORS FOR COLORECTAL CANCER RISK WITHIN QUARTILES OF DIETARY IRON AND SERUM IRON INDICES

Variable	Q1	Q2	Q3	Q4	*p* for trend
Dietary iron (mg/day)					
Quartile median (quartile cut-points)	12.2 (≤14.4)	16.8 (>14.4–18.6)	20.4 (>18.6–22.0)	25.0 (>22.0)	
Cases/controls	33/65	32/65	20/65	32/65	
OR[1] (95% CI)	1.0	1.0 (0.6–1.8)	0.6 (0.3–1.2)	1.1 (0.6–1.9)	0.88
OR[2] (95% CI)	1.0	0.8 (0.4–1.8)	0.5 (0.2–1.2)	0.4 (0.1–1.1)	0.06
Serum ferritin (ng/ml)					
Quartile median (quartile cut-points)	59 (≤88)	116 (>88–141)	181 (>141–221)	312 (>221)	
Cases/controls	40/68	29/64	36/64	25/64	
OR[1] (95% CI)	1.0	0.8 (0.4–1.5)	1.0 (0.6–1.8)	0.7 (0.4–1.3)	0.37
OR[2] (95% CI)	1.0	0.6 (0.3–1.2)	1.0 (0.5–1.9)	0.4 (0.2–0.9)	0.09
Serum iron (μg/dl)					
Quartile median (quartile cut-points)	83 (≤97)	109 (>97–124)	139 (>124–153)	175 (>153)	
Cases/controls	40/66	32/65	32/65	26/64	
OR[1] (95% CI)	1.0	0.8 (0.5–1.4)	0.8 (0.5–1.5)	0.7 (0.4–1.3)	0.28
OR[2] (95% CI)	1.0	0.8 (0.4–1.7)	0.8 (0.4–1.6)	0.7 (0.3–1.5)	0.41
Serum transferrin saturation (%)					
Quartile median (quartile cut-points)	25.9 (≤29.7)	33.4 (>29.7–37.6)	41.8 (>37.6–47.3)	53.4 (>47.3)	
Cases/controls	39/65	33/65	34/65	24/65	
OR[1] (95% CI)	1.0	0.8 (0.5–1.5)	0.9 (0.5–1.6)	0.6 (0.3–1.2)	0.17
OR[2] (95% CI)	1.0	0.9 (0.5–1.8)	1.0 (0.5–1.9)	0.6 (0.3–1.3)	0.24
Serum TIBC (μg/dl)					
Quartile median (quartile cut-points)	284 (≤300)	311 (>300–325)	338 (>325–351)	370 (>351)	
Cases/controls	27/66	33/64	35/66	35/64	
OR[1] (95% CI)	1.0	1.2 (0.7–2.2)	1.4 (0.7–2.6)	1.4 (0.7–2.5)	0.33
OR[2] (95% CI)	1.0	1.1 (0.5–2.3)	1.5 (0.7–3.2)	1.4 (0.7–3.2)	0.28
Serum UIBC (μg/dl)					
Quartile median (quartile cut-points)	150 (≤167)	186 (>167–200)	214 (>200–230)	255 (>230)	
Cases/controls	25/69	33/62	36/64	36/65	
OR[1] (95% CI)	1.0	1.6 (0.9–3.0)	1.7 (0.9–3.2)	1.6 (0.8–3.0)	0.21
OR[2] (95% CI)	1.0	1.6 (0.8–3.4)	1.5 (0.7–3.3)	1.7 (0.8–3.6)	0.25

[1]OR adjusted for age only.–[2]Multivariate adjusted OR: model includes age (continuous), education (junior high school and above versus less), body mass index (continuous, kg/m^2), number of years smoked, total number of cigarettes per day, physical activity (active—moderate or heavy occupational or recreational activity versus inactive), total caloric intake (kcal/day), alcohol (g/day of ethanol), ever daily aspirin use and serum CRP (continuous, mg/l).

TABLE IV – MULTIVARIATE-ADJUSTED ORS[1] FOR COLON (n = 73) AND RECTAL (n = 57) CANCER RISK WITHIN QUARTILES OF DIETARY IRON AND SERUM IRON INDICES

Variable	Q1	Q2	Q3	Q4	p for trend
Dietary iron (mg/day)[2]					
Colon cancer cases/controls	20/37	18/36	9/37	18/36	
OR (95% CI) for colon cancer	1.0	0.7 (0.3–2.0)	0.4 (0.1–1.4)	0.3 (0.1–1.6)	0.09
Rectal cancer cases/controls	15/29	12/28	11/29	14/28	
OR (95% CI) for rectal cancer	1.0	1.3 (0.4–4.4)	0.8 (0.2–3.8)	0.5 (0.1–2.8)	0.38
Serum ferritin (ng/ml)					
Colon cancer cases/controls	26/38	15/35	21/37	11/36	
OR (95% CI) for colon cancer	1.0	0.6 (0.2–1.5)	0.8 (0.3–2.1)	0.2 (0.1–0.7)	0.02
Rectal cancer cases/controls	14/29	12/30	17/27	14/28	
OR (95% CI) for rectal cancer	1.0	0.6 (0.2–1.9)	1.2 (0.4–3.6)	0.8 (0.2–2.9)	0.79
Serum iron (μg/dl)					
Colon cancer cases/controls	25/37	21/36	18/39	9/34	
OR (95% CI) for colon cancer	1.0	1.0 (0.4–2.5)	0.8 (0.3–2.1)	0.2 (0.1–0.9)	0.05
Rectal cancer cases/controls	14/29	13/28	14/30	16/27	
OR (95% CI) for rectal cancer	1.0	0.9 (0.2–3.6)	0.7 (0.2–2.4)	1.7 (0.5–6.1)	0.35
Serum transferrin saturation (%)					
Colon cancer cases/controls	27/37	22/36	16/37	8/36	
OR (95% CI) for colon cancer	1.0	0.7 (0.3–1.7)	0.4 (0.1–1.2)	0.1 (0.02–0.5)	0.003
Rectal cancer cases/controls	11/29	13/28	20/29	13/28	
OR (95% CI) for rectal cancer	1.0	1.2 (0.3–4.7)	2.5 (0.8–7.5)	2.3 (0.6–7.9)	0.12
Serum TIBC (μg/dl)					
Colon cancer cases/controls	16/37	19/37	16/36	22/36	
OR (95% CI) for colon cancer	1.0	1.1 (0.4–3.0)	1.6 (0.6–4.6)	1.9 (0.7–5.8)	0.19
Rectal cancer cases/controls	14/29	13/28	17/29	13/28	
OR (95% CI) for rectal cancer	1.0	0.9 (0.3–2.8)	1.5 (0.5–5.2)	0.8 (0.2–2.8)	0.92
Serum UIBC (μg/dl)					
Colon cancer cases/controls	11/37	17/36	21/37	24/36	
OR (95% CI) for colon cancer	1.0	1.6 (0.5–4.8)	2.0 (0.6–7.1)	4.7 (1.4–15.1)	0.009
Rectal cancer cases/controls	14/29	16/30	15/27	12/28	
OR (95% CI) for rectal cancer	1.0	1.6 (0.5–4.9)	1.0 (0.3–3.0)	0.4 (0.1–1.7)	0.22

[1]Multivariate adjusted ORs model includes age (continuous), education (junior high school and above versus less), body mass index (continuous, kg/m^2), number of years smoked, total number of cigarettes per day, physical activity (active—moderate or heavy occupational or recreational activity versus inactive), total caloric intake (kcal/day), alcohol (g/day of ethanol), ever daily aspirin use, and serum CRP (continuous, mg/l).–[2] Dietary iron information missing for 8 colon cancer cases and 5 rectal cancer cases.

Stratified analyses by dietary factors thought to affect iron absorption did not reveal any statistically significant associations (data not shown). A stratified analysis by anatomic sub-site revealed associations specifically for colon cancer. The highest quartiles, compared to the lowest, of serum ferritin, serum iron and transferrin saturation were all inversely associated with colon cancer risk (OR = 0.2, 95% CI = 0.1–0.7, p trend = 0.02; OR = 0.2, 95% CI = 0.1–0.9, p trend = 0.05; OR = 0.1, 95% CI = 0.02–0.5, p trend = 0.003, respectively) (Table IV). In contrast, serum UIBC was associated with an elevated risk for colon cancer (OR = 4.7, 95% CI = 1.4–15.1, p trend = 0.009). None of the measured iron indices were associated with rectal cancer (Table IV).

This ATBC study did not collect information on diagnoses of hereditary hemochromatosis, a condition resulting in excessive iron absorption. To ensure that unknown presence of this disease in the dataset was not influencing our findings, we conducted additional sensitivity analyses that excluded individuals in the top 1% (n = 18) and top 10% (n = 52) of serum ferritin, and these results did not differ from our main findings.

Discussion

The current study found a nonsignificant inverse association for dietary iron and colorectal cancer risk, and a significant inverse association for serum ferritin and colorectal cancer risk. Serum ferritin, serum iron and transferrin saturation were all inversely associated with colon cancer risk specifically, but not rectal cancer risk, whereas serum UIBC was associated with a greater risk for colon cancer.

Studies demonstrating that products of Fenton chemical reactions involving iron can be genotoxic[17] suggest that iron is associated with both the initiation as well as promotional phases of carcinogenesis, because of the increased iron requirement of cancer cells as a result of hyper-proliferation.[3,18] Furthermore, tumor cells have a greater ability than normal cells to grow and survive in the presence of high concentrations of iron[19]; when iron is deficient, the rate of tumor growth actually decreases.[20]

There are two distinct routes of exposure to iron. First, excess iron absorption can result in elevated iron in the circulation, which could result in deleterious effects at any organ site; however, iron absorption is generally tightly regulated. Complementing tightly controlled absorption, the second route of exposure can occur as a result of excess dietary iron passing through the gastrointestinal tract where it can exert deleterious effects in the lumen directly. Earlier studies have confirmed that unabsorbed iron is concentrated in feces at levels ten-fold higher than most tissues, therefore the potential formation of the toxic hydroxyl radicals through Fenton chemistry is greatly increased in the large bowel.[21–24]

There is controversy as to whether diet can dramatically influence iron stores. Experimental studies have reported that dietary iron cannot induce iron overload[25–27], although a more recent study in a large American cohort found a significant positive association between dietary factors, including meat, and increased iron stores.[28] Heme iron is more readily absorbed that nonheme iron and high heme iron intakes have been associated with high plasma ferritin concentrations.[29] Our study found a slightly elevated serum ferritin level in those who consumed higher amounts of red meat, a proxy for heme iron intake; the difference was not significant, however, which was probably a result of measurement error associated with using red meat variables as representative of heme iron consumption.

There have been conflicting findings with regard to dietary iron and colorectal cancer risk: studies have reported both no association[30] and positive associations.[9,31–33] We found no correlation between dietary iron (or intake of meat) and iron stores; this lack of association was expected in light of the degree to which iron

absorption is regulated, and because iron absorption is affected by many other factors (*e.g.* other dietary components able to enhance, such as ascorbic acid or inhibit absorption, such as phytate).[34,35] Furthermore, in this cohort of Finnish males, no association was found for red meat as a risk factor for colorectal cancer.[36] Unfortunately, we were unable to address whether supplemental iron was associated with colorectal cancer risk due to the small number of individuals reporting taking supplemental iron; only 3.1% of cases and 6.9% of controls reported iron supplement use.

Both null[11] and positive associations[9,10] have also been reported for indices of serum iron and colorectal cancer risk. The findings from our study complement the nested case-control study by Kato *et al*.,[37] that found an inverse association for serum ferritin and colorectal cancer risk in women; in addition, the inverse findings from that study and ours were both driven by colon cancer and not rectal cancer.

There are several mechanisms that could explain an inverse association between serum iron indices and colon cancer risk, either individually or in combination. Low serum iron indices may be indicative of less iron being absorbed and consequently more iron present in the gastrointestinal tract where oxidative damage may occur directly to the colorectal lumen. Furthermore, iron depletion may be evident in colorectal cancer cases due to the increased iron requirement during tumor growth.[3,18] Alternatively, this might reflect occult bleeding from a tumor; sixty percentage of newly diagnosed colorectal cancer cases exhibit signs of iron deficiency.[38] Although all cases in this study were diagnosed at least 5 years after the blood collection, it is still possible with the long induction period associated with colorectal cancer (approximately 5–10 years) that changes in serum iron indices due to disease may still have occurred prior to serum collection in the cases in this study.

The significant associations from this study were restricted to colon cancer cases. Earlier studies have found a distinct difference between the effects of iron on the colon compared with the rectum. Our findings replicate an association with colon cancer but not rectal cancer in one earlier study,[37] whereas others have reported effects of iron on cancers of the rectum and distal colon.[33,39,40] It has been suggested that colon and rectal cancers have different etiologies,[41] although it is not clear why iron would only exert effects in specific sub-sites.

This study has several noteworthy strengths. It employed a prospective design with long follow-up, which enabled us to exclude cancers diagnosed in the first 5 years of follow-up and reduce the potential for underlying disease-associated changes in baseline dietary and serum indices. Among the limitations, consideration must be given to the generalizability of data from a cohort of male Finnish smokers. We studied a relatively small number of cases and had only one baseline measurement of serum indices. In addition, the cases had not undergone formal cancer screening procedures, other than a chest X-ray, and therefore, it is likely that some individuals had sub-clinical colorectal neoplasia at baseline when the iron indices were measured; although we attempted to minimize this potential bias by formally excluding cases diagnosed within the first 5 years. Furthermore, there is wide interindividual variation in iron absorption and metabolism, which could be explained by polymorphisms in genes governing iron homeostasis. The relation between these variants and iron overload or deficiency has not been studied extensively, except for some studies investigating polymorphisms in the *HFE* gene, which is associated with hereditary hemochromatosis. Hereditary hemochromatosis is the most common cause of iron overload and alleles shown to underlie this condition have also been associated with increased risk of colon cancer.[42] Future work regarding iron as a risk factor for colorectal cancer should have sufficient power to incorporate a genetic component, as this may help clarify the associations and their mechanisms.

In summary, this nested case-control study of iron and colorectal cancer risk found a significant inverse association between several serum iron indices and colon cancer risk.

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